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SHORT COMMUNICATION

Effect of solvent-solvent partition on antioxidant activity and GC-MS profile of *Ilex guayusa* Loes. leaves extract and fractions

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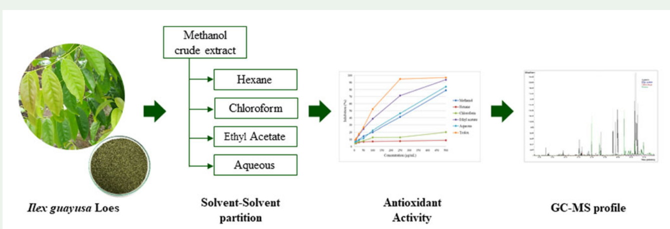
ABSTRACT

The use of guayusa (*Ilex guayusa* Loes.) leaves as functional food has increase recently. This work discusses the antioxidant activity and volatile compounds of guayusa leaves extract and fractions. The methanol crude extract was obtained by maceration, subsequently hexane, chloroform, ethyl acetate, and aqueous fractions were collected by solvent-solvent partition. Total phenolic content (TPC), total flavonol/flavone content (TFC), 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, and ferric reducing antioxidant power (FRAP) were measured by ultraviolet-visible (UV-Vis) spectrophotometry. The results revealed that ethyl acetate fraction showed highest inhibition against DPPH radical (93.86 ± 0.95%) at 500 µg/mL, and reduce the ferric-tripyridyltriazine complex (Fe^{3+}-TPTZ) at 1619.81 mg trolox equivalent (TE)/g, followed by aqueous fraction. This bioactivity could be related to phenolic acids, flavones and flavonols content, as well as the caffeine, dodecanoic acid isopropyl ester, caffeic acid, and malic acid identified by gas chromatography-mass spectrometry (GC-MS). These findings support the antioxidant properties of this plant material.

Graphical Abstract



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1. Introduction

Guayusa (*Ilex guayusa* Loes.) is a tropical evergreen tree belonging to the Aquifoliaceae family that grows in the amazon region of Colombia, Ecuador, Peru, and Bolivia. The folk medicine has informed that many Amazonian communities use guayusa leaves as diuretic, diaphoretic, emetic, hypnotic, narcotic, stimulant, and purgative (Radice et al. 2017). In Ecuador, guayusa leaves have been used for preparation of energy beverages, and to relief stomach pain by the Kichwa ethnicity, mestizo farmers, and rural population (Abril-Saltos et al. 2016). Nowadays, guayusa leaves have gained commercial value, and they have exported worldwide to be consumed as an herbal tea due to indigenous knowledge and chemical properties (Wise and Negrin 2020). Scientific findings have demonstrated the antioxidant, antiglycemic, antifungal, antibacterial, and anti-inflammatory effects of guayusa leaves extracts (Sequeda-Castañeda et al. 2016). Other studies have reported the concentration of caffeine, theobromine and theophylline in leaf alcoholic extracts, being caffeine the major alkaloid quantified (Negrin et al. 2019). In fact, the effect of drying methods has been assessed, and the convention oven showed the highest content of caffeine than air-drying, and solar drying (Manzano-Santana et al. 2018). Equally important, the carotenoids content of hydroalcoholic extracts have not shown significant differences between mature and young leaves (Villacís-Chiriboga et al. 2018), and some flavonoids have been quantified in green leaves and processed guayusa (García-Ruiz et al. 2017). Also, optimized conditions for extraction of phenolic antioxidants by ultrasonic bath have been obtained for guayusa leaves (Arteaga-Crespo et al. 2020). Nonetheless, bioactive compounds of guayusa capable of scavenging free radicals have been shortly reported. Therefore, the antioxidant activity and chemical profile using GC-MS of guayusa leaves were carried out in order to contribute supplementary phytochemical information about this plant species, which is used as a natural source of antioxidant compounds.

2. Results and discussion

2.1. Effect of solvent partition on antioxidant activity

The results summarized in Table S1 indicate that predominantly ethyl acetate fraction has shown the highest antioxidant inhibition values followed by the aqueous fraction, and methanol crude extract. TPC of methanol crude extract found in this study were higher than previous reported data for ethanol/water (34.6 mg GAE/g), and ethanol (22.33 ± 0.15 mg GAE/g) extracts obtained by ultrasonic bath (Arteaga-Crespo et al. 2020), and soxhlet extraction (Cadena-Carrera et al. 2019) respectively. Also, TPC of aqueous fraction is higher than a guayusa commercial product (198.36 ± 4.11 mg GAE/g) obtained after 60 minutes of brewing time (Pardau et al. 2017), and TPC of ethyl acetate fraction is greater than phenolic content informed for this solvent (7.64 ± 0.08 mg GAE/g) using soxhlet apparatus (Cadena-Carrera et al. 2019). Differences could be associated to the extraction technique used, since referenced studies employed temperature-assisted procedures, and degradation of thermolabile compounds might affect the availability of phenolic components (Santos-Buelga et al.

2012). Alternatively, TFC as quercetin equivalents has not been previously reported, and it considered only flavones and flavonols structures. Flavanones and dihydroflavonols were not expected since an alternative colorimetric technique has been proposed for their quantitation (Popova et al. 2004). TFC values of this work were higher than flavonols content reported mainly as quercetin-3-O-hexose for hydroalcoholic extracts of young (3.16 mg/g), and old (1.98 mg/g) guayusa leaves (Villacís-Chiriboga et al. 2018). In particular, TPC showed a positive linear correlation with the antioxidant activity against DPPH radical (r = 0.898) and ferric-tripyridyl-triazine complex (r = 0.989), as well as between TFC and the antioxidant activity against DPPH radical (r = 0.934) and ferric-tripyridyl-triazine complex (r = 0.969). These strong associations were statistically significant ($p < 0.05$), reveal dependency between response variables and suggest that scavenging activities could be explained by the presence of phenolic acids, flavones and flavonols. Data from the literature confirm the existence of such relationships (Pardau et al. 2017; Villacís-Chiriboga et al. 2018; Cadena-Carrera et al. 2019). Consequently, the crude extract, ethyl acetate and aqueous fractions showed higher inhibition against DPPH radical and the IC_{50} values found were greater than a methanol extract of guayusa leaves obtained by maceration: 14.2 ± 0.99 µg/mL (Jaramillo Fierro and Ojeda Riascos 2018). In fact, the higher concentration assessed of ethyl acetate fraction reveal similar inhibitory potential as Trolox standard (Figure S1). In addition, methanol crude extract, chloroform, ethyl acetate, and aqueous fractions showed higher FRAP values than data informed for guayusa leaf hydroalcoholic extract (0.080 mmol Trolox equivalents/100 g) (Arteaga-Crespo et al. 2020). In contrast, the hexane fraction did not manifest DPPH inhibition, FRAP reducing capacity and exposed the lowest total phenolic and flavonoid contents. These significance differences between samples could be attributed to solvents polarity and the hydrophilic reaction of antioxidant tests used. In consequence, the solvent-solvent partition used in this study was effective to separate bioactive compounds of crude extract soluble in non-polar and dipolar aprotic solvents.

2.2. GC-MS analysis

The identified compounds of fractions from guayusa leaves are shown in Table S2. In total 29 low weight compounds were detected: 5 free fatty acids, 3 benzene derivatives, 4 terpenes, 2 xanthines, 2 phytosterols, 2 tocopherols, 2 glycerides, 2 esterified fatty acids, 1 carboxylic acid, 1 hydroxycinnamic acid, 1 diterpenoid, 1 monoterpenoid, 1 polyol, 1 benzofuran, and 1 sugar alcohol. All compounds appear in order of elution of chromatographic column and they were confirmed by the n-alkane retention index (Figure S2). The most components were detected in hexane and aqueous fraction, while barely 3 compounds altogether were identified in chloroform and ethyl acetate fractions. Particularly, caffeine, dodecanoic acid isopropyl ester, and theobromine were found in these intermediate fractions. In fact, caffeine was the majority compound of this study, and it was detected in all fractions. This xanthine derivative was the main component of chloroform fraction (99.22 ± 0.01%), and ethyl acetate (77.10 ± 8.90%). In this context, the high antioxidant inhibition exposed by ethyl acetate fraction would probably be explained mostly to the presence of this alkaloid, and the existence of

dodecanoic acid isopropyl ester (3.59 ± 1.48%). Similarly, the good scavenging activity of aqueous fraction could be related mainly by the occurrence of caffeic acid (0.37 ± 0.06%), and malic acid (0.24 ± 0.01%). Although, the non-volatile composition of this fraction was not determined by this study. On the other hand, the hexane fraction presented the lowest antioxidant activity values. Nevertheless, some potential antioxidant compounds were identified: squalene (14.43 ± 0.48%), hexadecanoic acid (11.52 ± 0.08%), oleic acid (7.78 ± 0.49%), alpha-Tocopherol (4.90 ± 0.22%), alpha-Amyrin (4.28 ± 0.29%), beta-Sitosterol (3.28 ± 0.14%), beta-Amyrin (1.93 ± 0.93%), octadecanoic acid (1.73 ± 0.02%), and stigmasterol (1.55 ± 0.01%). In general, the presence of main compounds could explain the bioactivity of assessed fractions and they support the separation procedure employed.

3. Experimental

See supplemental material for standards and chemicals, extraction, antioxidant activity assays, and GC-MS procedure.

4. Conclusions

This work revealed that ethyl acetate, and aqueous fractions obtained by solvent-solvent partition showed high inhibitory potential against DPPH radical and ferric-tripyridyl-triazine complex. These scavenging activities exposed linear correlation with phenolic acids, flavones and flavonols content found. The bioactivity could be also explained by the presence of caffeine, dodecanoic acid isopropyl ester, caffeic acid, and malic acid. Hence, these findings contribute significant scientific data about guayusa leaves and suggest complementary studies focused on non-volatile compounds of hydrophilic extract or fractions from this plant material.

Disclosure statement

The authors have declared no conflict of interest.

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